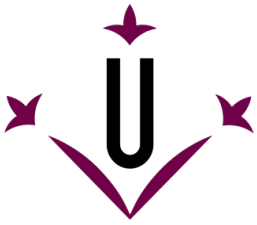

Title: Captures of oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae), in traps baited with host-plant volatiles in Chile

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Abstract

Studies in Australia and China identified host-plant volatile blends from peach and pear that captured relatively high numbers of *Grapholita molesta* (Busck). To determine if these blends are attractants in other countries and relative to each other, the two host-plant blends, a laboratory blend identified in Switzerland, and a new "Total blend" made by mixing components of all three blends, were field tested in Chile for the first time. Same solvent type, concentrations and dispensers as in the original studies, plus an additional concentration and solvent, were used. Only the Swiss blend at the low *n*-hexane concentration captured significantly more males than the solvent traps, yet, in very low numbers (1.46 ± 1.46, mean ± SEM males/trap/week). Furthermore, host-plant blends decreased male captures in sex pheromone traps, and the effect was dose-dependent for the Chinese and Total blends. A laboratory flight-tunnel test confirmed the lack of *G. molesta* male response to the Australian, Chinese and Swiss plant blends. In the flight tunnel, however, the males responded sooner and in higher numbers to mixtures of sex pheromone with host-plant blends than they did to the sex pheromone alone.

Keywords

host-plant volatiles, sex pheromone, synergism, flight tunnel, traps

Introduction

Moths rely on their sense of smell to locate mates. Females release relatively small quantities of highly volatile pheromone molecules detected by very sensitive receptors on the male antennae (Allison and Cardé 2016). The high sensitivity and species-specificity of moth sex pheromones and their strong effect on males has made them a cornerstone tool in moth pest control. Pheromones are used to monitor insect-pest occurrence, time insecticide applications, bring the insect in contact with insecticides, or remove a significant part of the population. Foremost, sex pheromones are used to disrupt mating and so reduce population levels and crop damage (Miller and Gut 2015; Witzgall et al. 2010). One way to determine if mating disruption negatively affects male attraction is to use sex pheromone traps to compare male captures in pheromone-disrupted and non-disrupted crops. However, not catching males with pheromone traps does not necessarily imply a failure of males to find females (Knight et al. 2013). In addition, pheromone traps attract only males, thus, no information is available on the mating status of females (Light et al. 2017). One method to facilitate monitoring of males and females in sex pheromone disrupted crops is to use host-plant volatile lures (Miller and Gut 2015). Despite the potential importance of plant volatiles in pest management, there are relatively few commercial plant volatile attractants for moth control (Szendrei and Rodriguez-Saona 2010). One of them is the pear ester, ethyl (*E*,*Z*)-2,4-decadienoate, a volatile from ripe pears that is a relatively selective attractant of male and female *Cydia pomonella* (L.) and is commercialized as a combo lure together with the pheromone for monitoring and increasing the efficiency of mating disruption (Knight et al. 2014; Light et al. 2017).

The oriental fruit moth, *Grapholita molesta* (Busck) is a worldwide pest of peach (*Prunus persica* (L.)), apple (*Malus domestica* Borkh), and other stone and pome fruit tree species (Rothschild and Vickers 1991). Early in the season the larva bores on green shoots, moving to new ones as they are consumed. This feeding hinders the formation of new branches and causes problems in tree nurseries. Later in the season, when shoots start to harden, feeding shifts to newly available fruit, which can cause major economic loss. *G. molesta* is primarily a pest of peach, but in recent years there have been increasing reports of damage to apple fruit worldwide (Wei et al. 2015). The selection of oviposition locations is thus vital and is aided, at least in part, by host-plant volatiles (Myers et al., 2007; Piñero and Dorn 2007).

The control of *G. molesta* relies strongly on repeated insecticide applications throughout the season, with the well-known negative impacts on humans and the environment (Guillette and Iguchi 2012). The release of the synthetic pheromone blends from passive dispensers or puffers results in mating disruption and population control (King et al. 2014, Witzgall et al. 2010). Although there are no commercial host-plant blends specific to monitor *G. molesta*, several field and laboratory studies show that host-plant-released volatiles elicit male and female *G. molesta* responses that are comparable to the natural host. In Australia, a synthetic volatile blend that mimics those emitted by young peach shoots captured up to 130 males per trap, although it did not capture females (Il'ichev et al. 2009). In China, several synthetic volatile blends mimicking pear (*Pyrus bretschneideri* Rehder and *Pyrus pyrifolia* (Burm.), and peach (*Prunus persica* (L.)) fruit and shoots have been identified (Lu et al. 2012, 2014, 2015). The volatile blend

obtained from pear (*P. bretschneideri* var. Jimi) fruit captured about 50 males and 20 females per trap. In the case of males, this was only 5 times less than what commercial sex pheromone traps captured in that study (Lu et al. 2012). In addition, this blend resulted in approximately 80% approach and 10% source contact by males in the flight tunnel; which was equivalent to the response of the natural fruit (Lu et al. 2012). In another study in Switzerland, analysis of peach shoot volatiles resulted in a blend that in dual-choice olfactometer tests, was as attractive to mated females as the natural host-plant blend (Piñero and Dorn 2007).

Captures of *G. molesta* in the Australian and Chinese studies was remarkable and paralleled the attraction of *C. pomonella* to the pear ester (Light et al. 2001). Thus, these new blends could be an invaluable tool for the management of *G. molesta*. However, *G. molesta* is a widely distributed species with significant genetic differentiation among world populations (Kirk et al. 2013), and therefore it is crucial to determine if the Australian and Chinese blends, which have been tested only in these two countries, are also attractants in other areas of the geographical distribution of this species. In addition, it would be useful to determine if the two blends are equally attractive. The Swiss blend, which attracts females under laboratory conditions, remains to be tested in the field. The first objective of our study was to compare the attractiveness of the Australian, Chilean and Swiss blends and to test them at a new location. The three plant blends, plus a new "Total" blend made with all the different components from the other three test blends, were tested together in a peach orchard in Chile, a country where *G. molesta* was first recorded in 1970 (González 2003). The second objective of this study was to explore the

potential of the host-plant blends to increase captures of males in sex pheromone traps, as this could improve the monitoring of males under mating disruption (Yu et al. 2014). To this end, we compared captures in traps baited with the pheromone alone and traps baited with the pheromone combined with the host-plant volatiles. Previous flight-tunnel studies show that the Chinese blend attracts males and females (Lu et al. 2012), and the Swiss blend does not attract males but synergizes their response to the sex pheromone (Varela et al. 2011). Our third objective was to compare responses between field and laboratory settings. To do so, the plant blends were tested in the flight tunnel alone and in combination with the sex pheromone.

Materials and Methods

Chemical stimuli

Host-plant volatiles were purchased from Sigma-Aldrich (Santiago, Chile, chemical purity, product and lot numbers in Table 1). The composition of the host-plant blends followed those reported by the Australian (Il'ichev et al. 2009), Chinese (Lu et al. 2012), and Swiss (Piñero and Dorn 2007) studies (Table 2). The Australian study used (*E*)-β-farnesene and (*E*)-β-ocimene, but pure isomers were not available to us at the time of the study, so a mixture of farnesene isomers and β-ocimene isomers were used instead (Table 1). A fourth host-plant blend made by combining the components from all three blends was included in the tests ("Total" blend, Table 2). To duplicate Australian and Chinese studies, all chemicals were dissolved in *n*-hexane and loaded in rubber septa (red, i.d x o.d., 3.4 mm x 6.6 mm, Sigma-Aldrich, Santiago, Chile, product number Z565709), at approximately

similar concentrations as in the original studies (100 mg total and 100 mg major compound, respectively, Table 3). The Swiss blend was prepared the same way as the Chinese blend (100 mg major compound), and the Total blend was made with 10 mg of each compound. Plant odor loads for all four blends ranged between 100 and 189 mg (Table 3). With the aim of providing a wider range of release rates, in experiment 1 (see below) the host-plant blends were prepared at an additional 10-fold lower concentration, and both high and low concentrations were further dissolved in mineral oil (Sigma-Aldrich product number M8410, lot number MKBG7544V, CAS number 8020-83-5) and loaded in 1.5 mL microcentrifuge tubes fitted with a 15-mm-long x 7-mm-diameter section of dental cotton wick to absorb the chemicals. Mineral or paraffin oil is a mixture of *n*-alkanes and provide slower and more linear release rates than individual shorter alkanes, like *n*-hexane, and both are used routinely to deliver plant volatiles in olfactory tests (Andersson et al. 2012). The lids of the microcentrifuge tubes were perforated with a 1.5-mm diameter hole and were kept closed. Rubber septa were rinsed in *n*-hexane and then in acetone, and allowed to dry, before use. Microcentrifuge tubes were rinsed in acetone. Host-plant volatiles were loaded in 500 μl volumes in rubber septa and microcentrifuge tubes at the concentrations indicated below. It took about 1 h for the host-plant blends to be absorbed by the rubber septa. For 2 to 4 h the dispensers were maintained in a well ventilated area and then placed inside plastic bottles and stored at -20C° until taken to the field on the following day.

In the Chinese study (Lu et al. 2012) commercial sex pheromone rubber septa were used. In our study the three sex pheromone components, (*Z*)-8-dodecenyl acetate (*Z*8-

12:Ac), *E*8-12:Ac, and (*Z*)-8-dodecenol (*Z*8-12:OH) (Pherobank, Wageningen, The Netherlands, > 99% pure) were diluted in *n*-hexane at a 100:5.4:10 ratio, respectively (Knight et al. 2015), and loaded in red rubber septa. In the first experiment we used 80 μg, which is an optimal quantity (Knight et al. 2015). Captures were relatively high so in experiments 2 and 3 we reduced the quantity of pheromone to 16 and 8 µg, respectively. This reduction in pheromone load permitted to test potential synergistic effects of host-plant volatiles on sex pheromone attraction.

Field tests

Experiments were carried out in a peach (*Prunus persica* var. *persica* cv. Doctor Davis and Carson) orchard in Chile (Duao, Maule, 35°33'29''S, 71°33'44''W) between December 21st 2012 and February 25th 2013 (Table 3). The Chinese and Australian studies used standard delta traps and funnel-type Efekto fly traps, respectively (Il'ichev et al. 2009, Lu et al. 2012). We used white delta traps (215 mm long x 200 mm wide x 100 mm tall, 340 cm^2 adhesive base area, Plastic Delta Trap, Alphascent, West Linn, OR, USA), except for the pheromone treatment in experiment 1, where the traps were red due to a temporary shortage of white traps. This should not have influenced trap catches because trap color does not affect *G. molesta* captures (Zhao et al. 2013). Traps were placed at 1.7 m high, hanging from 4-cm-diameter blue PVC pipes fitted in the tree branches. Traps within a plot were placed in a transect 15 to 20 m apart, and plots were at least 15 m apart from each other. Trap floors were lined with removable sticky cards.

In experiment 1 the dispensers, either rubber septa or microcentrifuge tubes, were hung from the ceiling of the trap with a wire, almost touching the trap floor. In the other two experiments the dispensers were placed directly on the sticky floor. Septa and microcentrifuge tubes were labeled with the treatment name using permanent markers. Traps lured with sex pheromone and host-plant odors (experiments 2 and 3) had 2 septa, one for each stimulus type, which were placed within a few cm of each other at the center of the trap. Sticky bottoms were replaced if there were captures. Sex of captured individuals was determined in the laboratory using a stereo microscope.

Experiment 1 was carried out between December 21st 2012 and January 29th 2013 and tested the 4 host-plant blends at two doses with two solvents and dispensers (Table 3). Sex pheromone (80 μg) and solvent (*n*-hexane or mineral oil) were the positive and negative controls, respectively, and were loaded in the corresponding dispensers (septum or microcentrifuge tubes). The 20 plant-volatile treatments [(4 plant treatments x 2 doses + solvent + pheromone) x 2 dispenser types (septum or microcentrifuge tube)] were placed in each of 4 rows, or plots, at random. There were 6 weekly trap checks. Sex pheromone and host-plant lures were replaced on the first and second checks. For the remainder of the experiment the pheromone lure was unchanged, while new plant lures were replaced one last time on the 4th check (January 11th 2013).

Experiment 2 was ran between January 29th and February 12th 2013 to determine if the host-plant blends had any effect on male attraction to the sex pheromone. Traps were baited with a sex pheromone septum (16 μg) and a host-plant blend septum at a similar dose as in the original Australian and Chinese studies, which was the same as the "high"

dose of experiment 1. This dose resulted in pheromone: host-plant volatile ratios ranging between 1:1,650 (Australian blend) and 1:11,812 (Chinese blend) (Table 3). Sex pheromone-only traps served as positive controls and *n*-hexane-only traps served as negative controls. The 10 treatments (4 host-plant blends; 4 sex pheromone + host-plant blends; solvent; sex pheromone) were replicated in 8 plots at random. There were 5 trap checks every 3 to 4 days. Sex pheromone septa were not replaced, and new plant lures were replaced on February 5th 2013.

Experiment 3, which was carried out between February 12th and 25th 2013, tested if the inhibitory effect of the host-plant blends observed in experiment 2 (see Results) was dose-dependent. It included the two plant blends that caused the strongest inhibition in experiment 2 (Chinese and Total, see Results), and sex pheromone at 8 μg. The pheromone:plant ratio ranged from the lowest 1:175 (Total) to the highest 1:23,625 (Chinese) (Table 3). Two host-plant volatiles, terpinyl acetate and β-ocimene isomer mix, which have shown behavioral activity in previous studies (Cichón et al. 2013; Il'ichev et al. 2009; Knight et al. 2014), were tested alone at 3 mg, as in Knight et al. (2014), and in combination with sex pheromone at a sex pheromone: host-plant volatile ratio of 1:375 (Table 3). In addition, we tested the effect of having one or two septa in the trap, by adding a blank septum to a trap with a pheromone septum. *n*-Hexane septa were tested alone to control for possible sex pheromone contamination. The 13 treatments (2 host-plant blends with sex pheromone x 3 doses; sex pheromone alone; sex pheromone and *n*-hexane septum; *n*-hexane; 2 host-plant volatiles with and without sex pheromone) were placed in 4 plots at random. There were 6 checks every 1 or 2 days because there was a

population peak during this period. Pheromone-loaded septa were not replaced, yet new host-plant lures were replaced on February 18th and 22nd 2013.

Flight tunnel test

The colony of *G. molesta* used in the flight tunnel was established with insects collected at Piacenza, Italy. The population has been maintained at the University of Lleida, Spain, since 2005 without reintroduction of wild individuals. Larvae were reared on a semi-synthetic diet modified from Ivaldi-Sender (1974) under a L16:D8 photoperiod at 24 ± 1°C. Pupae were separated by sex and placed in 4-L polypropylene containers and provided a cotton ball soaked in 10% sugar water solution. Adults were collected daily and used when 2 to 4 days old.

The flight tunnel and its methodology have been previously described (Ammagarahalli et al. 2017). The 150 x 45 x 45 cm (length x height x width) tunnel had a 0.35 m s^{-1} wind flow and the temperature was maintained at 23 ± 1°C. It was illuminated from above with 36-watt fluorescent lamps producing 150 lux white light. Tests were carried out during the last 3 hours of the photophase and occasionally into the first hour of the scotophase, in which case the daylight illumination was left on. Males were placed individually in glass tubes and were transferred to the flight tunnel room 30 to 120 min before the beginning of the test. Test odors were applied in 10 µl loads to 10 x 15 mm filter paper pieces that were allowed to dry for 5 to 10 min until tested in the flight tunnel 5 to 180 min later. The male was placed in the flight tunnel after the odor stimulus, on top of a metal-wire platform similar to the one used for the odor source and 1.3 m downwind

from it. We recorded for 2 min if the male took flight, started upwind oriented flight (zigzagging upwind flight) or landed on the filter paper containing the stimulus source, and the time it took the males to engage in these behaviors. The stimulus was placed in the tunnel on top of a 25-cm-tall metal-wire platform. Three to five males flew to each filter paper treatment before changing to another treatment paper. At the end of a test day a filter paper had been used with 8 to 15 males, therefore, the filter papers were outside their individual glass vial and exposed to the wind flow between of 32 to 60 min before being discarded. On any given day, only one filter paper was used for each treatment. Due to the high number (see below), the treatment order was randomized in two groups and tested on alternate days.

The following treatments were tested in the flight tunnel: Australian, Chinese and Swiss host-plant blends at 10 μg each, a suboptimal sex pheromone dose of 1 ng (a response curve to doses of 1, 10, 100 and 1,000 ng resulted in 34, 82, 89 and 63% source contact respectively, N = 44, similar to Ammagarahalli et al. (2017)), and sex pheromone: host-plant blends (Australian, Chinese or Swiss) at 1:10, 1:100, 1:1,000, and 1:10,000 ratios with sex pheromone at 1 ng (Table 3). We used a suboptimal dose of sex pheromone because the optimal dose results in a high percentage of response. The 10 μg dose of host-plant odors was 50 times lower than that used in the Chinese flight tunnel test with rubber septa (Lu et al. 2012), yet similar to what we have used previously (Varela et al. 2011). To control contamination, 20 insects were tested with *n*-hexane on random days. The sex pheromone:host-plant blends were prepared using a stock sex pheromone solution so all had identical sex pheromone concentrations. The 1:0, 1:100, 1:1,000 and

1:10,000 blends were prepared on January 18th, 2013 and the 1:10 blend on February 4th, 2013. Flight tunnel tests were carried out between February 8th and 27th, 2013 with N = 64.

Statistical analyses

Generalized linear models (GLM) with Poisson family function in the package lme4 of R (R Development Core Team 2015) were used to analyze trap count data (Bolker et al. 2009). Due to the high temporal variation in trap captures, sampling date was included as a random effect in the generalized linear mixed model (GLMM), along with the variation among plots if they contributed significantly to the model after comparing among models with ANOVA. To treatments with zero captures, a random capture was added so the GLMM could converge. The percentage of males which responded in the flight tunnel was analyzed with GLM models using a binomial family function. Behavioral categories (take flight, oriented flight and contact) were analyzed separately. One response was added to treatments with no responses in a randomly chosen replicate so the GLM model could converge. The time elapsed before insect response inside the flight tunnel was analyzed with a linear model, lm(), in transformed [log (x+1)] data. Comparisons among treatment pairs in both field and flight tunnel studies were performed with the glht() or lsmeans() functions of R using Tukey´s alpha correction method. Whenever the term "significant" is used in the text regarding treatment comparisons it indicates that $p \leq 0.05$. Raw data and R scripts are available at https://repositori.udl.cat/handle/10459.1/59534

Results

Field tests

In experiment 1 only one female was captured in a trap baited with a high dose of the Swiss host-plant blend diluted in mineral oil. The four traps baited with sex pheromone septa captured a total of 1,632 males, whereas, the four traps baited with sex pheromone microcentrifuge tubes captured a total of 215 males. All of the host-plant volatile traps combined, which summed 64, captured a total of 64 males in the entire experiment (Table 4). Of these 64 males, 61 were captured in the 4th weekly check, and these captures clustered mainly in 3 particular traps: 35 males in a trap baited with the low-dose Swiss host-plant blend in *n*-hexane, 15 males in a trap baited with the high-dose Chinese host-plant blend in mineral oil, and 9 males in a trap baited with a high-dose Total host-plant blend in *n*-hexane. This level of captures in host-plant baited traps was not observed before or after week 4 (only 3 more males were captured by host-plant-baited traps in the other 5 weekly checks) or in experiment 2 (see below). Table 4 summarizes total trap captures, and shows how the Swiss host-plant blend at the low dose in *n*-hexane was the only host-plant blend that captured significantly more males (1.46 ± 1.46 males/trap/check, mean ± SEM), than the *n*-hexane traps, which captured none.

In experiment 2, traps baited with host-plant volatiles or with *n*-hexane captured no males, whereas sex pheromone-baited traps captured many males (2,800 in total, Table 5). Only 9 females were captured in this experiment, all of them in traps baited with the Australian host-plant blend combined with the sex pheromone, but these captures were not significantly different than those from the *n*-hexane traps. The addition of a

septum baited with any host-plant blend to a trap baited with a sex pheromone septum significantly decreased the number of males captured with respect to sex pheromone traps. This negative effect was significantly stronger for the Chinese and Total host-plant blends than for the Australian and Swiss host-plant blends.

In experiment 3, a total of 13,650 males and 17 females were captured. The addition of a septum baited with any of the three doses of the Chinese or Total host-plant blends to a trap baited with a sex pheromone septum significantly decreased the number of males captured relative to traps baited with a sex pheromone septum and an *n*-hexane septum. This effect was more pronounced as the host-plant dose increased (Table 6). Traps baited with a sex pheromone septum and an *n*-hexane septum captured more males than traps baited with only the sex pheromone septum. *n*-Hexane traps captured 6 males in total.

β-ocimene (mixture of isomers) captured significantly more males than *n*-hexane traps. Terpinyl acetate added to sex pheromone significantly increased captures relative to traps baited with a sex pheromone septum, but not relative to traps baited with a sex pheromone and a *n*-hexane septum together (Table 6).

The 17 females caught in experiment 3 were captured by 8 different treatments, 5 of which captured only one female, while 3 captured more than one female. The treatments that captured more than 1 female were always a combination of the host-plant blend with the sex pheromone. Of these, the highest captures were in β-ocimene (mix of isomers) plus sex pheromone which captured 3 females in one plot on three different dates, 3 females in another plot on two different dates, and one female in

another plot. None of the female captures in these treatments were significantly higher than *n*-hexane traps.

Flight tunnel test

In the flight tunnel experiment several doses of the Australian, Chinese and Swiss host-plant blends were added to sex pheromone. None of the host-plant blends alone, nor *n*-hexane, attracted any males, so they were not included in the means comparison test. Pairwise comparisons between each sex pheromone host-plant combination treatment and the isolated sex pheromone treatment showed that the three host-plant blends significantly increased the percentages of flight, oriented flight and contact, and did so in a dose-dependent manner (Fig. 1a). The Chinese and Swiss host-plant blends significantly increased responses at the 1:100 to 1:1,000 sex pheromone:host-plant ratios, whereas the Australian host-plant blend did so at the 1:1 and 1:10 ratios. The three host-plant blends significantly reduced the time of response to the sex pheromone, and, as with the percentages of response, the effect was stronger at the higher (plant wise) sex pheromone:host-plant odor ratios (1:1,000 and 1:10,000) (Fig. 1b).

Discussion

Captures by the Australian and Chinese host-plant blends in our study were substantially lower than in the original studies (from here onwards "Australian and Chinese studies" will refer to "Il'ichev et al. 2009 and Lu et al. 2012", respectively). Although the experimental conditions of the Australian and Chinese studies were closely reproduced in Chile, there

were some differences that perhaps account for the low response of these host-plant blends in our experiments. The most evident difference is the chemical purity of farnesene and ocimene, which in the Australian study consisted of (*E*)-β-farnesene and (*E*)-β-ocimene. The isolated isomers were not commercially available at the time of the tests, therefore, a mixture of isomers of each compound were used in our study. Insects can distinguish odorant isomers both at the sensory and behavioral levels (De Bruyne and Baker 2008), so it is possible that isomeric purity affected reproducibility of the Australian blend in Chile. However, the β-ocimene mix of isomers in our study was one of the few plant stimuli that was more attractive than *n*-hexane, whereas the Australian blend influenced male response to pheromone, as did the other plant blends. Although our Australian blend did not have the same isomer purity as the original Australian blend, our results show that it was sensed by and affected the behavior of *G. molesta*. Furthermore, the Chinese blend used a mixture of farnesene isomers both in the original Chinese study and in our study. Even though it was very attractive in China, it performed very poorly in Chile. Therefore, isomer purity should not have contributed to the different performance of the Chinese blend in China and Chile. Little is known about the detection of plant volatiles in *G. molesta*, but olfactory receptor neurons relatively specific to the same farnesene isomer mix that we used in here have been described on the antenna of males (Ammagarahalli and Gemeno 2015).

Although we used the same solvents, concentrations and dispensers as in the original Australian and Chinese studies, the quantity and proportion of odorants released by the dispensers were not analyzed in any of these studies. Due to this we do not know if

the volatile composition and emission rate would have varied among them. By using an additional lower concentration than in the original Australian and Chinese studies, and mineral oil as an extra solvent, we attempted to diversify the stimulus quantity released by the dispensers. Mineral oil probably decreased release rates because pheromone in mineral oil attracted about 7-times fewer males than pheromone in *n*-hexane. A similar solvent effect was probably true for the host-plant volatiles, however neither mineral oil nor the lower stimulus concentration improved captures relative to the original solvent and concentration. In our tests, dispensers with plant volatiles were loaded a day before used and replaced every 5.6 ± 3.9 days (mean ± SEM) in order to minimize stimulus degradation and depletion during the assay. A final methodological difference among studies was the use of a funnel-type fly trap in the Australian study, whereas in the Chinese and Chilean studies standard delta traps were used. Although trap type could affect moth captures, our sex pheromone traps captured many males, thus it is unlikely that trap type alone could explain the dissimilar performance of the Australian blend in Australia and Chile. With regard to the flight tunnel test, we used a lower host-plant odor concentration than the Chinese study, and loaded it on filter paper instead of rubber septa, so the difference between the lack of response in our flight tunnel test and the good response in the Chinese flight tunnel test could be related to the use of different stimulus delivery conditions.

Biological variables such as population genetics could also be involved in the differences between the Australian and Chinese studies and our Chilean test. When populations evolve host-specialization, differences in the detection of host-plant volatile

signals may arise (Smadja and Butlin 2009). Significant genetic differences among populations of *G. molesta* could provide the necessary genetic diversity for the evolution of host varieties (Zheng et al. 2015; Wei et al. 2015). Nonetheless, host-plant specialization may be negligible in *G. molesta* given its recent human-aided expansion and reduced dispersal power (Wei et al. 2015). This is consistent with the lack of worldwide variation in sex pheromone production and response that we have reported in this species (Knight et al. 2015). However, whether this is also true regarding responses to host-plant volatiles remains to be tested.

Background odors in the environment could influence the response of insects to odor stimuli (Cai et al. 2017). Pear ester is more effective in attracting *C. pomonella* when deployed in walnut orchards than in pear orchards (Light et al. 2001). In China, pear- and peach-odor blends perform relatively better in orchards of the opposite host (Lu et al., 2014; 2015). Captures with a pear blend in a peach orchard were >40 males per trap (Fig 2A in Lu et al. 2015). With this in mind we expected substantial captures of *G. molesta* in our peach fields with the Chinese pear blend, but captures were never higher than in control traps. The Australian peach blend was very attractive in a pear background (Il'ichev et al., 2009), so its poor performance in our peach orchard might be related to the differences in host-plant backgrounds. Yet, in China the peach blends attract *G. molesta* in peach orchards (>10 males/trap, Fig 2A in Lu et al. 2015), and the pear blend is equally effective in peach and pear orchards (Fig 2B in Lu et al., 2012), so the relative importance of background odors needs to be examined. Background odors, however, could be relatively important to explain differences between laboratory and field studies. Knudsen

et al. (2008), for example, show the host-plant volatile compound which attracts the apple fruit moth, *Argyresthia conjugella* Zeller, in the flight tunnel is different than the one that attracts it in the field, albeit these compounds are released by the host. The authors conclude that the interaction of the plant volatiles with the background odor contributes to different results between field and laboratory tests. The flower volatile phenylacetaldehyde is a generalist noctuid moth attractant (Tóth et al. 2010) that increases the response of male *Spodoptera frugiperda* Walker to sex pheromone in the flight tunnel (Meagher and Mitchel 1998). However, this flower volatile decreases captures in sex pheromone traps in the field (Meagher 2001). Perhaps the lack of background plant odors in our flight tunnel test could explain why males were more attracted to the pheromone plus host-plant lures than to the pheromone lure alone. However, it seems unlikely that the presence of background plant odors in the field is responsible for the inhibitory effect of the host-plant blends on sex pheromone traps.

An important advantage of host-plant volatiles over sex pheromones is the potential attraction of females, which provides information on the mating status of the population. The Australian study reports that only males were attracted to the plant lures (Il'ichev et al. 2009), and the Chinese study reports male:female ratios in the range of 10:1 to 3:1 (Lu et al. 2012). In our study the plant odor blends also caught substantially more males than females (122 vs 2 in total, respectively). Interestingly, 24 additional females (92% of all the females caught in the study) were collected in traps baited with a combination of sex-pheromone and host-plant septa. Given recent reports of pheromone autodetection by female moths, and its possible implications in mating disruption

(Holdcraft et al. 2016), it may be worth exploring if the sex pheromone plays any role on female moth attraction to host-plant lures.

A field study with *G. molesta* shows that two plant volatiles [(*Z*)-3-hexenyl acetate and undecanol] added to the sex pheromone synergize captures at a 1:0.5 pheromone:host-plant ratio, but at the 1:1 and 1:2 ratios the synergism disappears, with a clear trend to become inhibitory at even higher host-plant ratios (Yu et al. 2014). In other moth species where synergism of host-pant volatiles with sex pheromone has been reported, the pheromone:host-plant ratio tested is in the range of 1:1 (e.g., Dai et al. 2008; Dickens et al. 1993; Light et al. 1993), although sometimes it can be as high as 1:40 (Deng et al. 2004). We used much higher sex pheromone:host-plant ratios, around 1: 10,000, in order to maintain the same host-plant concentrations as in the original Australian and Chinese studies. But when we later tested a lower (1:200) ratio in the dose-response test (experiment 3), the effect was also inhibitory relative to traps baited with a sex pheromone and an *n*-hexane septum. Thus, it remains to be determined if still lower sex pheromone:host-plant ratios of the Australian and Chinese plant-blends could have a non-inhibitory, or even a synergistic effect on sex pheromone captures. There are at least two other cases where host-plant odors reduce moth captures in sex pheromone traps. (*E*)-2-Ppenten-1-ol reduced captures of the arctiid moth *Hyphantria cunea* (Drury) when added to the sex pheromone at a 1:1 ratio (Tang et al. 2012), and phenylacetaldehyde reduced pheromone captures of *Spodoptera frugiperda* (Smith) in traps baited with a commercial sex pheromone septum and different amounts of the host-plant odorant (Meagher 2001).

Increased captures by the presence of a blank septum in sex pheromone traps was unexpected, and could be a visual response because *G. molesta* flies during the last light hours of the day using visual information (Kuenen and Gilbert 2014). It could also be a response to increased air turbulence, a factor that facilitates male upwind flight (De Bruyne and Baker 2008). Barros-Parada et al. (2016) report a large effect of the location of pheromone and pear ester septa and acetic acid dispensers in the trap on captures of *C. pomonella*. Our observations highlight the importance of taking into account apparently negligible experimental variables, like the addition of a second septum, which could impact trap captures and hamper the correct evaluation of host-plant attractants.

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Fig. 1 Effects of adding various host-plant volatile blends (Australian, Chinese and Swiss) of different doses on the behavioral responses of *G. molesta* males to a suboptimal dose of sex pheromone in a flight tunnel (experiment 4). a) Percentage of males engaged in each behavioral category (take flight, oriented upwind flight and source contact). b) Time it took males to engage in each behavioral category. Asterisks indicate significant differences between the sex pheromone host-plant combination treatments and the isolated sex pheromone treatment (1:0) by means of planned pairwise comparisons using Tukey`s test ($p \leq 0.05$)

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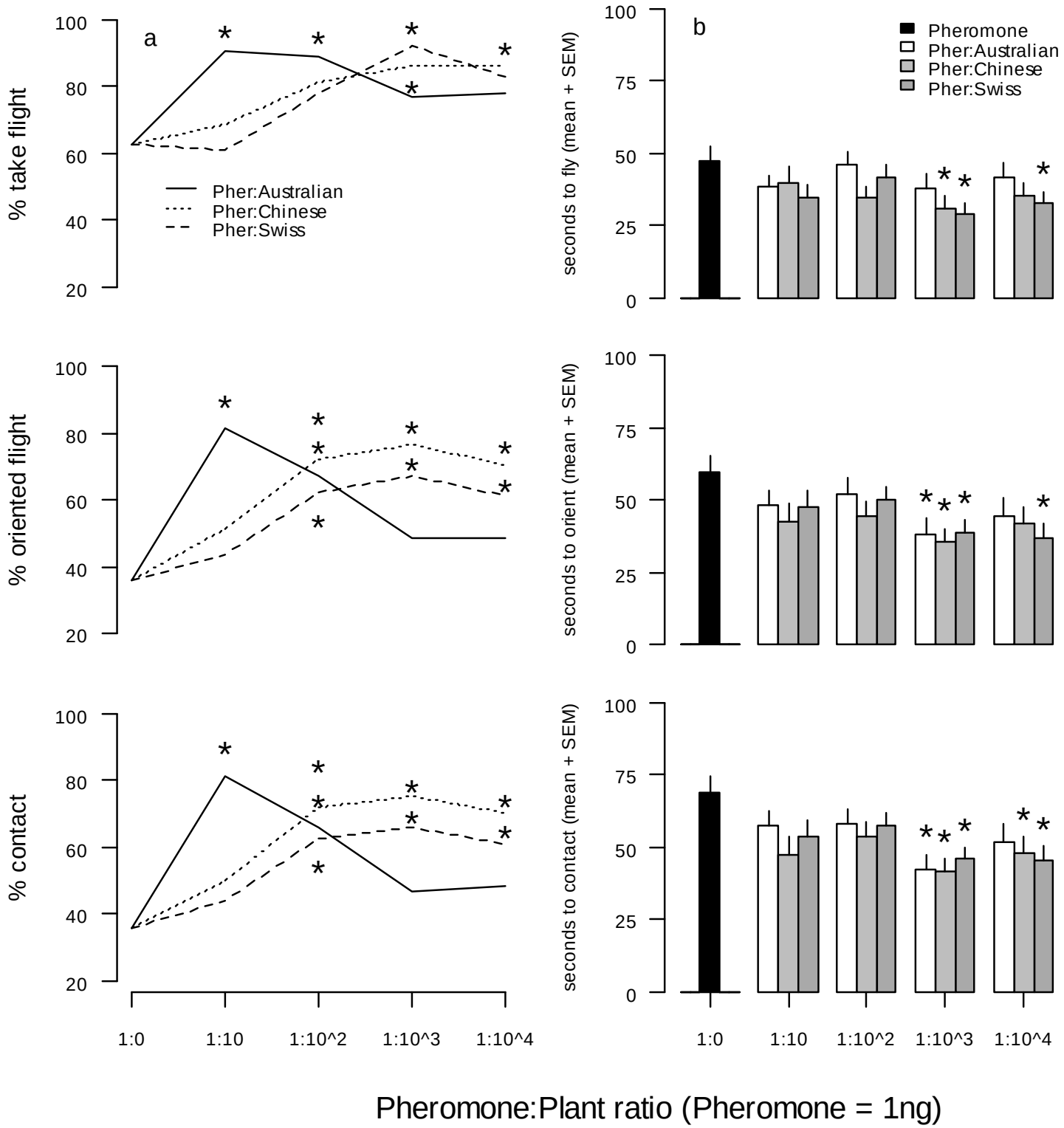
Figure


a
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Pher:Swiss
Pheromone
Pher:Australian
Pher:Chinese
Pher:Swiss
1:0
1:10
1:10^2
1:10^3
1:10^4
Pheromone:Plant ratio (Pheromone = 1ng)

Table 1 Synthetic host-plant odorants used in the experiments

Compound	CAS	Product number (Sigma Aldrich)	Lot number	Purity[a] (≥ %)
1-Hexanol	111-27-3	H13303	STBC8538V	98
Nonanal	124-19-6	W278203	STBC3506V	95
Ethyl butanoate	104-54-4	E15701	STBB7416V	99
Butyl acetate	123-86-4	402842	SHBB8826V	99.5
Ethyl hexanoate	123-66-0	148962	S28172V	99
Hexyl acetate	142-92-7	108154	STBC6608V	99
Hexyl butanoate	2639-63-6	W256803	STBC0651V	98
Farnesene[b]	NA	W383902	MKBG4494V	NA
(*Z*)-3-Hexenyl acetate	3681-71-8	W317101	MKBG6087V	98
(*Z*)-3-Hexenol	928-96-1	W256307	MKBG7249V	98
(*E*)-2-Hexenal	6728-26-3	W256005	STBC8608V	95
Benzaldehyde	100-52-7	B1334	STBC6885V	99
Benzonitrile	100-47-0	12722	BCBH8265V	98
β-Ocimene[b]	13877-91-3	W353901	MKBK5322V	90
Pear ester	3025-30-7	W314803	STBC4363V	80
Terpinyl acetate	80-26-2			95

[a] As indicated by manufacturer
[b] Mixture of isomers

Table 2 Odorant ratios in the four tested host-plant blends. Actual quantities used in the tests are shown in Table 3

Plant compound	Host-plant blend name			
	Australian[a]	Chinese[b]	Swiss[c]	Total
1-Hexanol		1		1
Nonanal		1		1
Ethyl butanoate		100		1
Butyl acetate		70		1
Ethyl hexanoate		7		1
Hexyl acetate		5		1
Hexyl butanoate		1		1
Farnesene[1]	100	4		1
(*Z*)-3-Hexenyl acetate	50		100	1
(*Z*)-3-Hexenol			20	1
(*E*)-2-Hexenal			3	1
Benzaldehyde			20	1
Benzonitrile			0.5	1
β-Ocimene[2]	100			1

[1] The Australian study used (*E*)-β-farnesene and the Chinese study used a mixture of farnesene isomers. We used a mixture of farnesene isomers
[2] The Australian study used (*E*)-β-ocimene, and we used a mixture of β-ocimene isomers
[a] Il'ichev et al., 2009
[b] Lu et al., 2012 (JM blend, Table 2)
[c] Piñero and Dorn, 2007

Table 3 Field and flight tunnel experimental details

Exp.	Objective	Start-end dates	Sex pheromone	Pheromone:host-plant ratio
1	Do host-plant blends attract *G. molesta*? Compare host-plant blends alone, at two doses and in two dispenser types (mineral oil in microcentrifuge tube vs *n*-hexane in rubber septum)	December 21, 2012 - January 29, 2013	80*µ*g	(Host-plant blends tested alone, two doses, in *n*-hexane or in mineral oil) Australian: 100 mg and 10 mg Chinese: 189 mg and 19 mg Swiss: 143 mg and 14 mg Total: 140 mg and 14 mg
2	Is there sex pheromone and host-plant synergism? Compare sex pheromone and host-plant blends at one host-plant dose.	January 29 - February 12, 2013	16*µ*g	Pher.:Australian, 1: 6,250 Pher.:Chinese, 1: 11,812 Pher.:Swiss, 1: 9,062 Pher.:Total, 1: 8,750 (Host-plant alone same as high conc. of exp. 1)
3	Is there sex pheromone and host-plant inhibition? Is inhibition dose-dependent? Compare sex pheromone and host-plant blends at several host-plant doses. Test additional host-plant compounds	February 12 - 25, 2013	8*µ*g	Pher.:Chinese, 1:237.5, 1:7,875, 1:23,625 Pher.:Total, 1:175, 1:5,825, 1:17,500 Pher.: β-Ocimene, 1:375 Pher:Terpinyl acetate, 1:375
4	Test sex pheromone and host-plant blends of field experiments in the wind tunnel	February 8 - 27, 2013	1ng	Pher.:Australian/Chinese/Swiss, 1:10, 1:100, 1:1,000, 1:10,000 (Plant alone: 10 µg)

Table 4 Captures of *G. molesta* in Chile between December 21st 2012 and January 29th 2013 in traps baited with one of 4 host-plant blends in two doses and either dissolved in *n*-hexane and loaded in red rubber septa, or dissolved in mineral oil and loaded in microcentrifuge tubes (Experiment 1)~~Solvents and sex pheromone were negative and positive controls, respectively. Only 1 female was captured in the entire experiment. Different letters within the *Males* columns indicate significant differences among treatments (Tukey, $p \leq 0.05$)~~

	Captures/trap/check (mean ± SEM)			
	n-Hexane in rubber septum		Mineral oil in microcentrifuge tube	
Stimulus	Males	Females	Males	Females
n-Hexane	0 [c]	0	0 [b]	0
Sex pheromone 80 µg	68.00 ± 13.99 [a]	0	8.96 ± 3.26 [a]	0
Australian 10 mg	0 [c]	0	0 [b]	0
Chinese 19 mg	0.08 ± 0.08 [c]	0	0 [b]	0
Swiss 14 mg	1.46 ± 1.46 [b]	0	0.04 ± 0.04 [b]	0
Total 14 mg	0 [c]	0	0 [b]	0
Australian 100 mg	0 [c]	0	0 [b]	0
Chinese 189 mg	0.04 ± 0.04 [c]	0	0.62 ± 0.62 [b]	0
Swiss 143 mg	0 [c]	0	0 [b]	0.04 ± 0.04
Total 140 mg	0.38 ± 0.38 [c]	0	0.04 ± 0.04 [b]	0

Solvents and sex pheromone were negative and positive controls, respectively. Only 1 female was captured in the entire experiment. Different letters within the *Males* columns indicate significant differences among treatments (Tukey, $p \leq 0.05$)

Table 5 Captures of *G. molesta* in Chile between January 29th and February 12th 2013 in traps baited with either, one septum of one of 4 host-plant blends, a sex pheromone septum, or sex pheromone with a host-plant blend septum (Experiment 2)~~Stimulus dissolved in *n*-hexane, tested as a negative control. Different letters within the male column indicate significant differences among treatments (Tukey, $p \leq 0.05$). Female captures by the Australian host-plant blend and *n*-hexane not significantly different~~

Stimulus		Captures/trap/check (mean ± SEM)	
Septum 1	Septum 2	Males	Females
n-Hexane		0 c	0
Sex pheromone 16 *µ*g		25.62 ± 3.27 [a]	0
Australian 100 mg		0 [d]	0
Chinese 189 mg		0 [d]	0
Swiss 143 mg		0 [d]	0
Total 140 mg		0 [d]	0
Australian 100 mg	Sex pheromone 16 *µ*g	20.41 ± 3.05 [b]	0.28 ± 0.11
Chinese 189 mg	Sex pheromone 16 *µ*g	10.28 ± 1.51 [c]	0
Swiss 143 mg	Sex pheromone 16 *µ*g	20.66 ± 2.18 [b]	0
Total 140 mg	Sex pheromone 16 *µ*g	10.53 ± 1.69 [c]	0

Stimulus dissolved in *n*-hexane, tested as a negative control. Different letters within the male column indicate significant differences among treatments (Tukey, $p \leq 0.05$). Female captures by the Australian host-plant blend and *n*-hexane not significantly different

Table 6 Captures of *G. molesta* in Chile between February 12th and February 25th 2013 in traps baited with one sex pheromone septum and a host-plant blend septum (Chinese or Total) at low, medium or high doses (Experiment 3)~~Stimulus dissolved in *n*-hexane, tested as a negative control. Terpinyl acetate and β-ocimene (isomer mix) tested alone or with a sex pheromone septum. A trap baited with sex pheromone and an *n*-hexane septum tested the effect of septum number. Different letters within the male column indicate significant differences among treatments (Tukey, $p \leq 0.05$). Female captures were not significantly different from *n*-hexane~~

Stimulus		Captures/trap/check (mean ± SEM)	
Septum 1	Septum 2	Males	Females
n-Hexane		0.21 ± 0.16 ^{h}	0.04 ± 0.04
Sex pheromone 8 µg		46.68 ± 6.35 ^{bc}	0
Sex pheromone 8 µg	*n*-Hexane	66.18 ± 8.48 ^{a}	0
Chinese 1.9 mg	Sex pheromone 8 µg	48.71 ± 9.83 ^{b}	0.04 ± 0.04
Chinese 63 mg	Sex pheromone 8 µg	39.75 ± 5.42 ^{d}	0
Chinese 189 mg	Sex pheromone 8 µg	24.57 ± 5.14 ^{ef}	0.04 ± 0.04
Total 1.4 mg	Sex pheromone 8 µg	40.71 ± 7.00 ^{cd}	0
Total 46.6 mg	Sex pheromone 8 µg	28.57 ± 4.37 ^{e}	0.07 ± 0.05
Total 140 mg	Sex pheromone 8 µg	21.46 ± 5.33 ^{f}	0.04 ± 0.04
Terpinyl Ac. 3 mg		0.57 ± 0.28 ^{gh}	0.04 ± 0.04
Terpinyl Ac. 3 mg	Sex pheromone 8 µg	63.82 ± 8.26 ^{a}	0.14 ± 0.07
β-Ocimene 3 mg		1.46 ± 1.39 ^{g}	0
β-Ocimene 3 mg	Sex pheromone 8 µg	52.93 ± 7.14 ^{b}	0.21 ± 0.08

Stimulus dissolved in *n*-hexane, tested as a negative control. Terpinyl acetate and β-ocimene (isomer mix) tested alone or with a sex pheromone septum. A trap baited with sex pheromone and an *n*-hexane septum tested the effect of septum number. Different letters within the male column indicate significant differences among treatments (Tukey, $p \leq 0.05$). Female captures were not significantly different from *n*-hexane